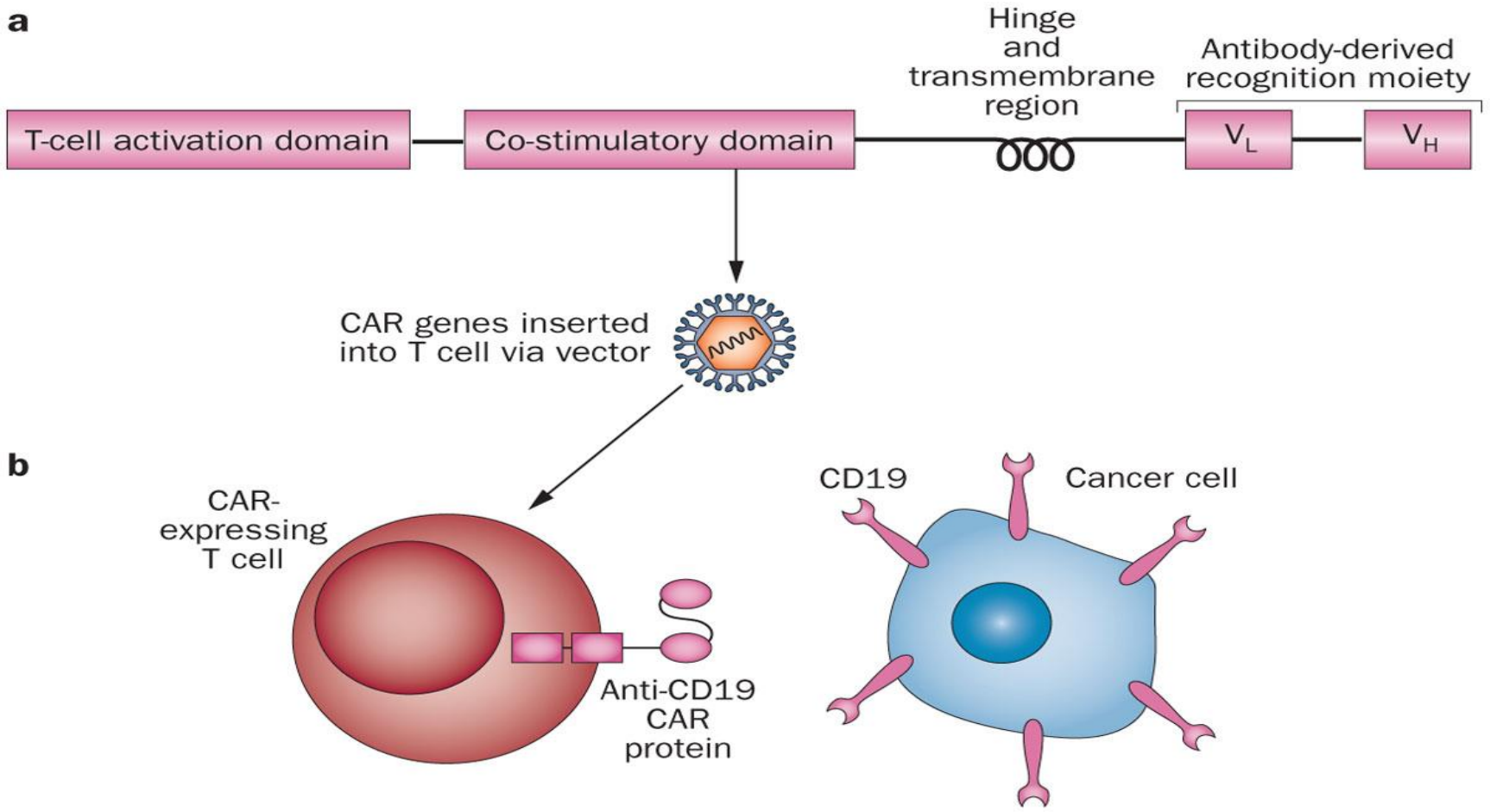
Chimeric Antigen Receptor T cell Therapy for Hematologic Malignancies

Lekha Mikkilneni, M.D.
P.I.: James N. Kochenderfer
Surgery Branch
National Cancer Institute

Anti-CD19 Chimeric Antigen Receptors (CARs)



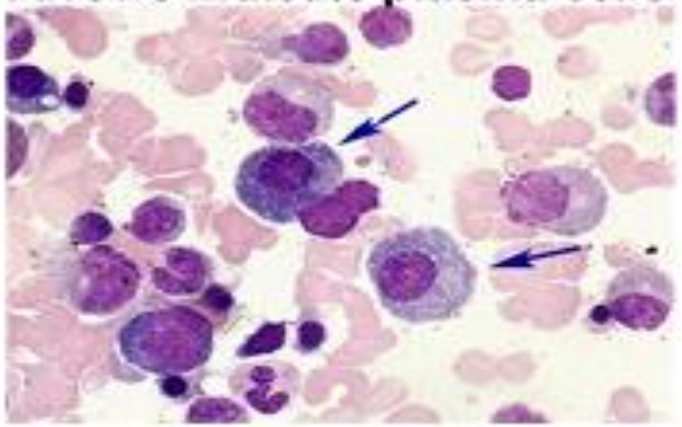
Kochenderfer, J. N. & Rosenberg, S. A. (2013)
Nat. Rev. Clin. Oncol.

B-cell maturation antigen

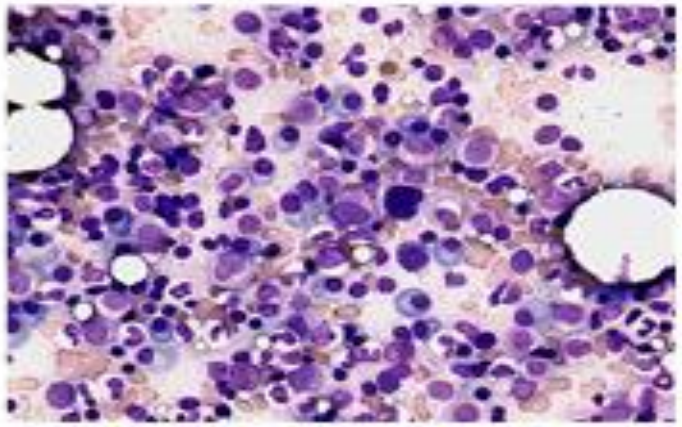
Multiple myeloma

Multiple myeloma

Arrows indicate plasma cells



Bone marrow with multiple myeloma



Sclerotic lesions of bones

CAR targeting BCMA

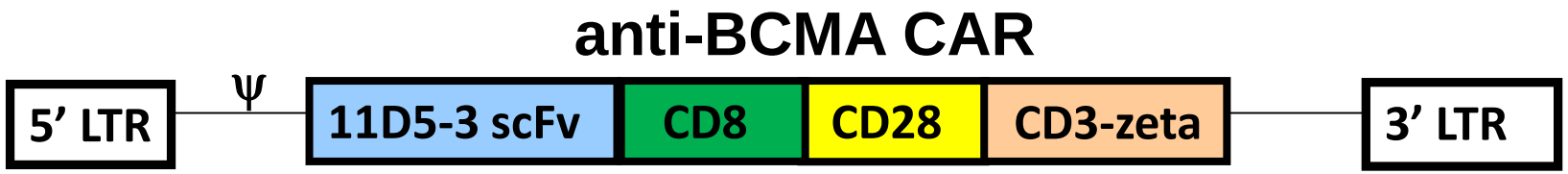
Development of the first CAR targeting B-cell maturation antigen (BCMA)

- BCMA (CD269) is a member of the TNF superfamily.
- By flow cytometry, BCMA is expressed on the myeloma cell surface by almost all cases of multiple myeloma.
- 34 different tissues were assessed by immunohistochemistry, BCMA was only expressed by plasma cells and a small fraction of B cells.
- We designed and tested the first series of anti-BCMA CARs

Carpenter et al. *Clinical Cancer Research*, 2013

T cells can be genetically engineered to express an anti-BCMA chimeric antigen receptor

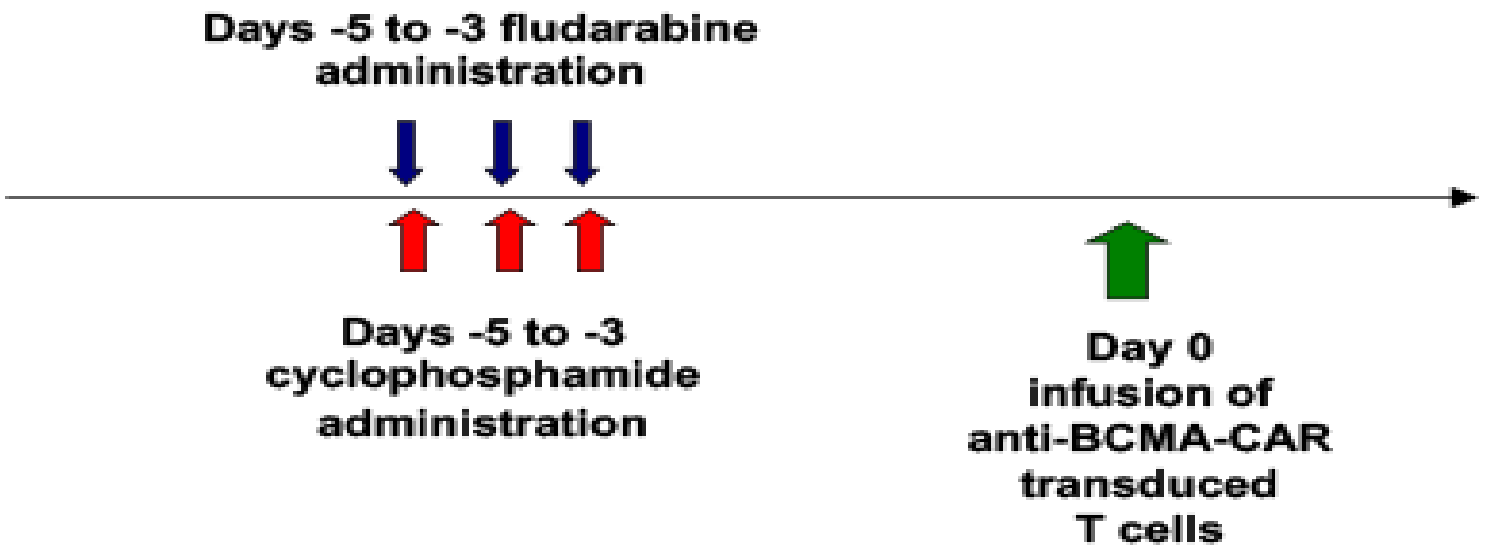
- We designed an anti-BCMA CAR and ligated it into a gamma-retroviral backbone.
- T cells were stimulated with the anti-CD3 monoclonal antibody OKT3 before transduction and cultured for 9 days before infusion.
- We initiated the first-in-humans clinical trial of an anti-BCMA CAR in 2014



Ali et al. *Blood,* 2016

Anti-BCMA CAR

Anti-BCMA CAR clinical protocol design



Cyclophosphamide: 300 mg/m² daily for 3 days

Fludarabine: 30 mg/m² daily for 3 days

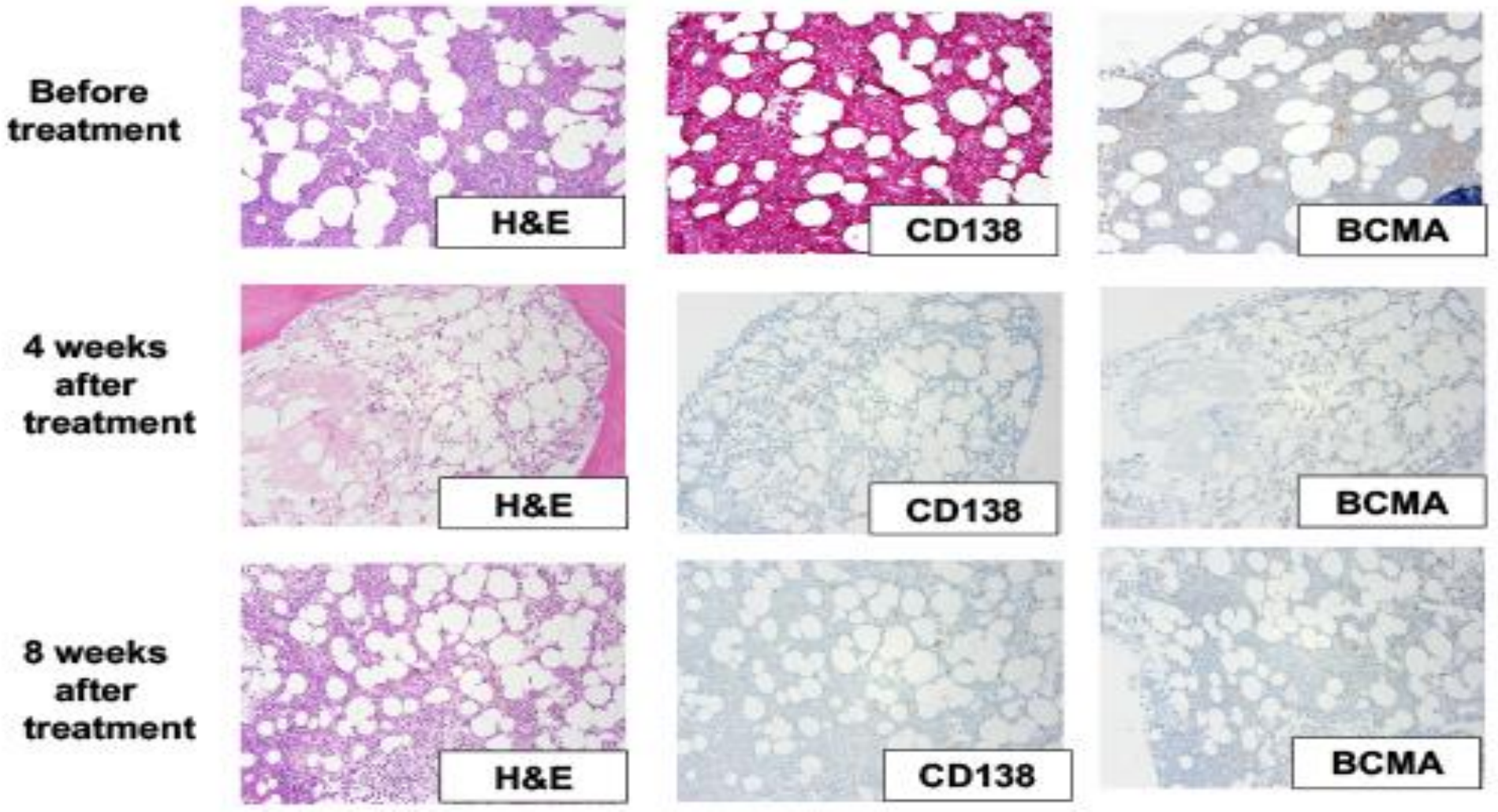
Baseline patient characteristics

Baseline characteristics of patients

- 24 patients treated on study; 2 patients received 2 cell infusions
- Median of 9.5 prior lines of therapy
- 6/15 evaluable patients (40%) with high risk cytogenetics, 5/15 (33%) with deletion 17p
-
- 10/16 patients (63%) refractory to last treatment regimen
- Patients treated on lower dose levels had very similar baseline characteristics as patients treated on highest dose level.

Bone marrow cells

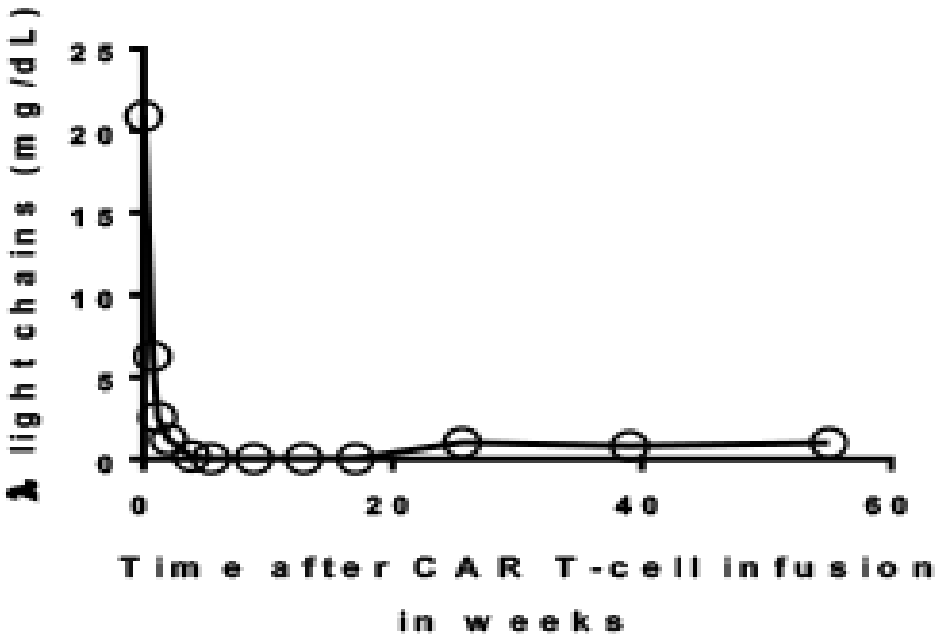
Multiple myeloma that made up more than 90% of Patient 10's bone marrow cells was eliminated after CAR T-cell infusion



Patient 14

Patient 14 attained VGPR of heavily pretreated extramedullary light chain myeloma

- 65 year old male with extramedullary λ light chain multiple myeloma
- Received 16 prior lines of therapy, including 2 autologous stem cell transplants
- He had a rapid decrease of λ light chains after CAR T-cell infusion
- His response was a VGPR that lasted 84 weeks.



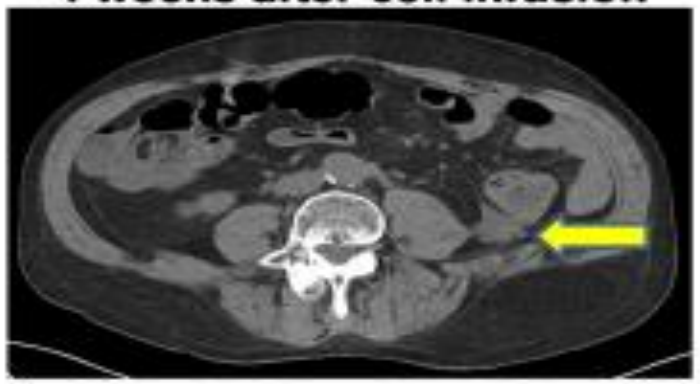
Brudno et al. *Journal of Clinical Oncology* 2018

Elimination of plasmacytoma

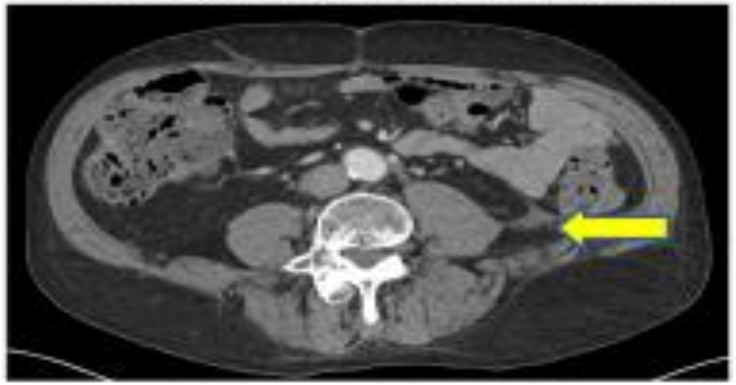
Elimination of soft-tissue plasmacytoma by anti-BCMA CAR T cells in Patient 14

Prior to cell infusion

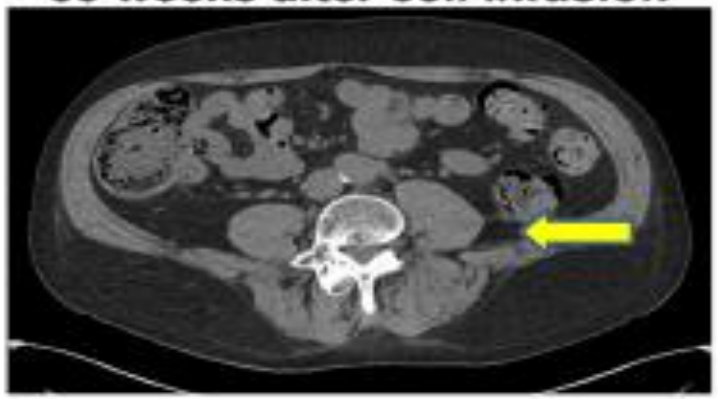
4 weeks after cell infusion



9 weeks after cell infusion



55 weeks after cell infusion



Anti-BCMA CAR T doses

Summary of responses of anti-BCMA CAR T at all dose levels

CAR T-cell dose/kg	Response (duration in weeks, + means ongoing)
$0.3x10^6$	PR (2), SD (6), SD (6)
$1x10^6$	SD (12), SD (4), SD (2)
$3x10^6$	SD (7), VGPR (8), SD (16), SD (2)
$9x10^6$	Stringent CR (17), VGPR (66), VGPR (29), VGPR (84), SD (2), VGPR (11), Stringent CR (69), VGPR (34), PR (31), VGPR (82), PD, VGPR (11), sCR (88), PR (2*), PR (29), SD (1)

Patients received no anti-myeloma therapy after infusion of CAR T cells until progression occurred

*Lost to follow-up

Brudno et al. *Journal of Clinical Oncology* 2018

Toxicity

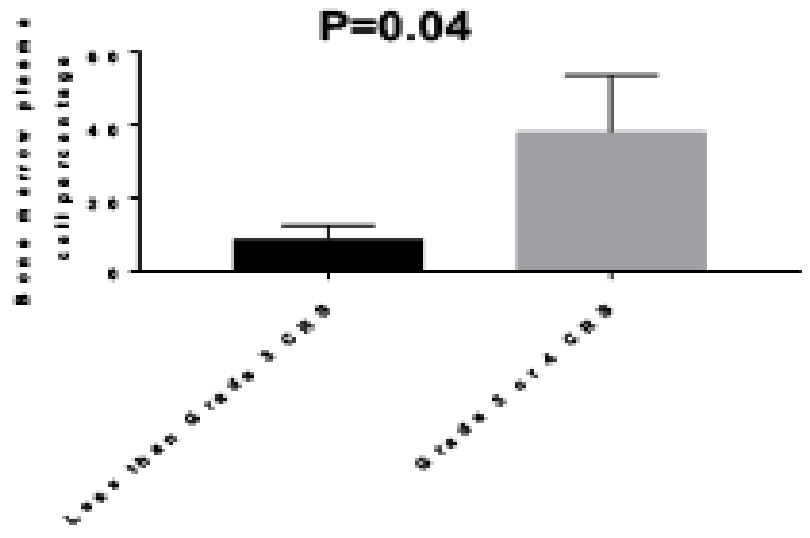
Toxicity of anti-BCMA CAR T cells: cytokines and myeloma burden

Cytokine release syndrome (CRS) on highest dose level (n=16):

- 2 patients with Grade 4
- 4 patients with Grade 3
- 10 patients with <Grade 3 CRS

Immunosuppression for CRS management:

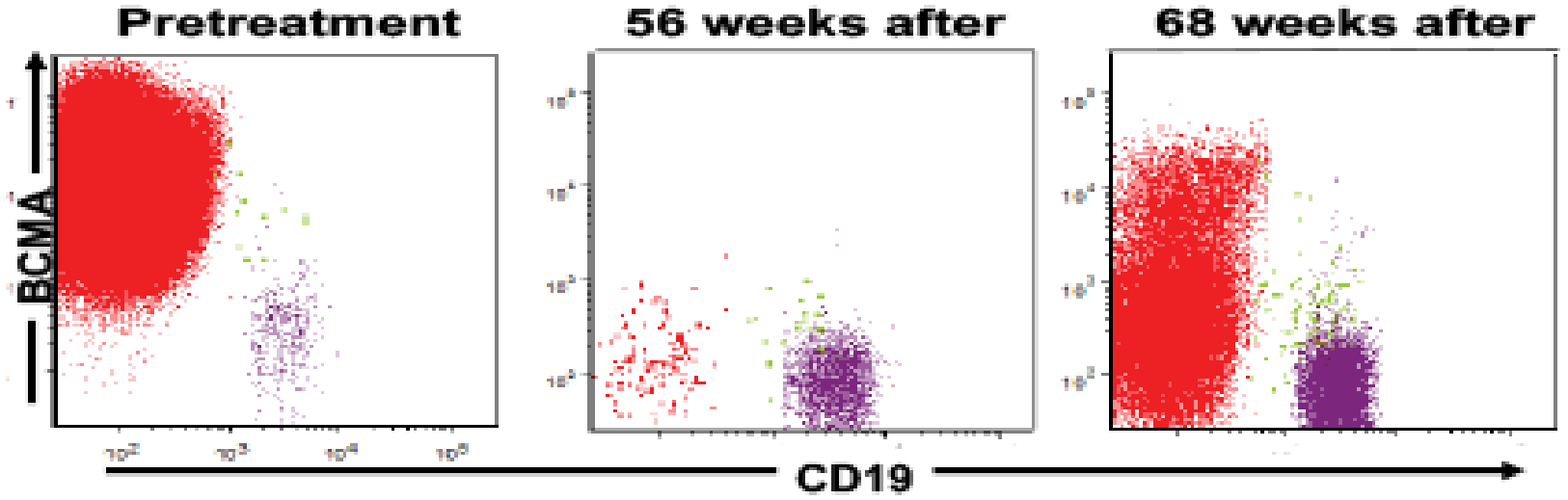
- 5 patients (31%) received tocilizumab for CRS management
- 4 of the patients who received tocilizumab also received corticosteroids for CRS management or adrenal insufficiency



Plasma cells

BCMA-negative plasma cells appeared in bone marrow 56 weeks after treatment followed by appearance of a mix of BCMA-positive and negative plasma cells

- Before treatment, bone marrow showed uniform BCMA expression on the CD19-negative plasma cells (red) and BCMA-negative CD19+ B cells (purple).
- The patient obtained a long response with no evidence of bone marrow myeloma
- A BCMA-negative population of malignant plasma cells appeared 56 weeks after treatment.



Brudno et al. *Journal of Clinical Oncology* 2018

Summary

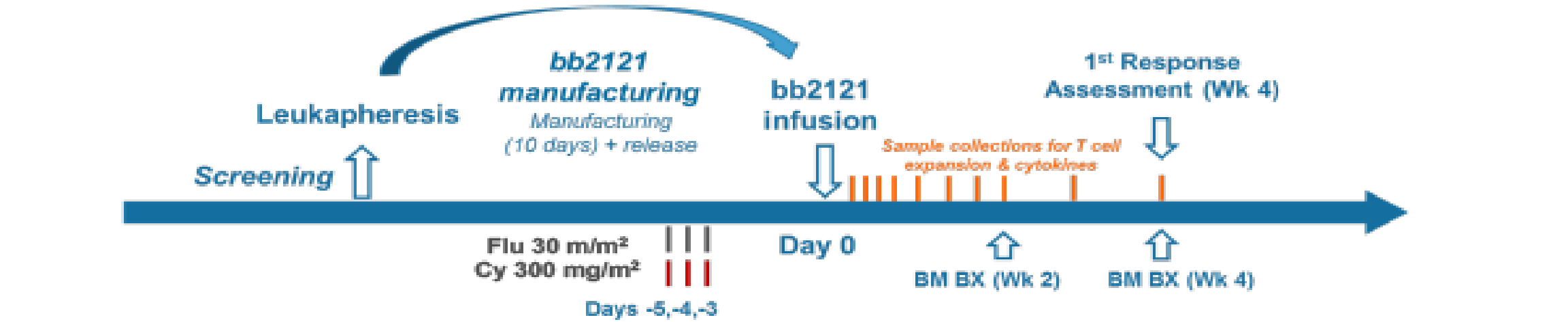
Summary of anti-BCMA CAR T cells at NCI single-center study

- Only 2/10 objective responses on dose levels 1-3
- 13/16 objective responses at optimal dose of $9x10^6$/kg (81% ORR)
- 5 of 16 patients on the optimum dose level have had durations of response of >1 year; 9/16 patients on the optimal dose had responses of >6 months
- Responses allowed patients to be off-therapy for many months
- Multiple myeloma is difficult to treat because of its phenotypic heterogeneity

Patients with multiple myeloma

bb2121 Anti-BCMA CAR T-cell therapy in patients with relapsed/refractory multiple myeloma: updated results from a multicenter phase I study CRB401

- The CAR used in bb2121 had the same 11D5-3 scFv as the previously mentioned CAR used at the NCI.
- The bb2121 CAR had a 4-1BB costimulatory domain and was encoded by a lentivirus



Dose Escalation (N=21)

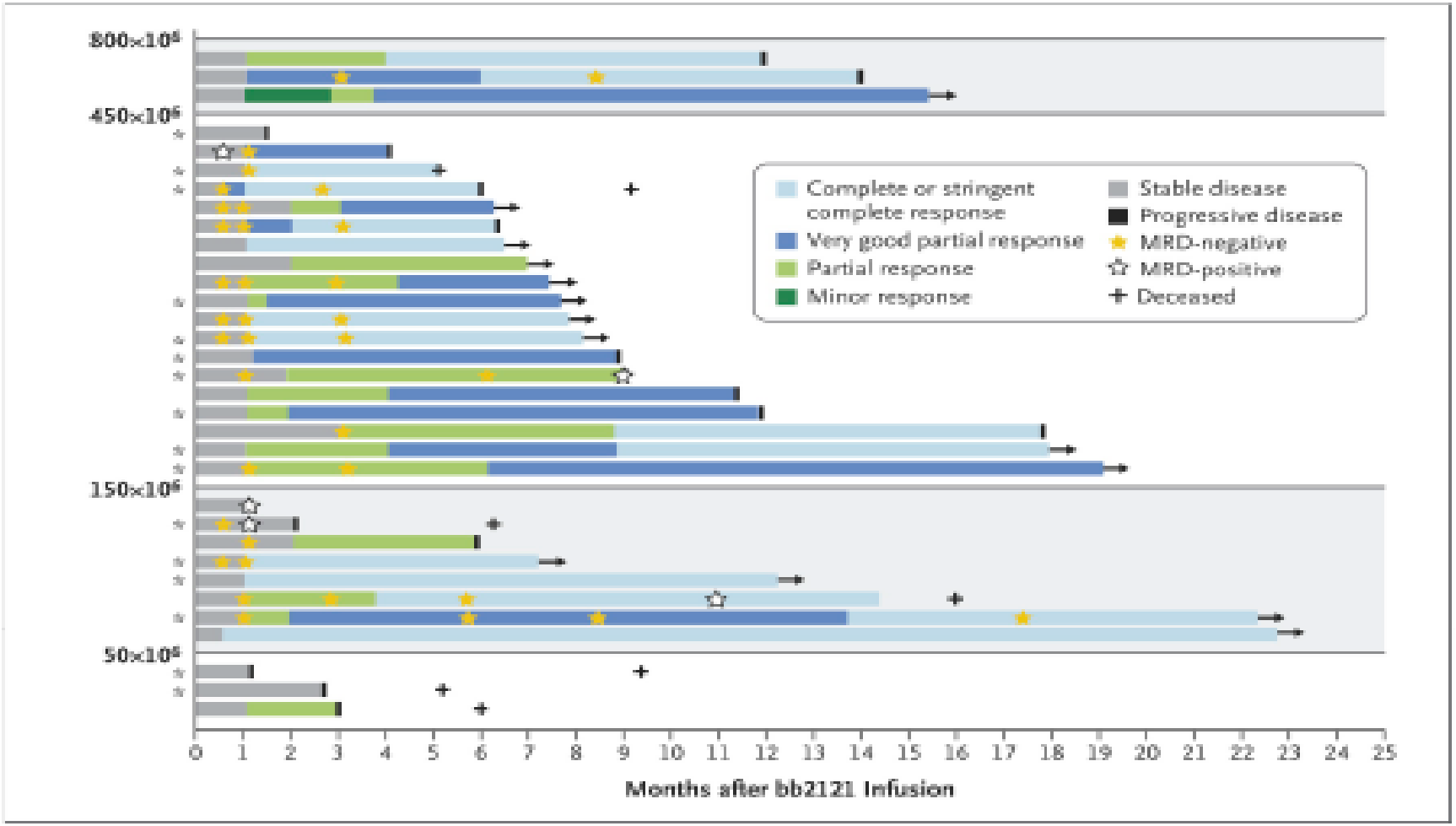
(median 7 prior anti-myeloma regimens)

Dose Expansion (N=22)

(median 7 prior anti-myeloma regimens)

Bb2121 responses

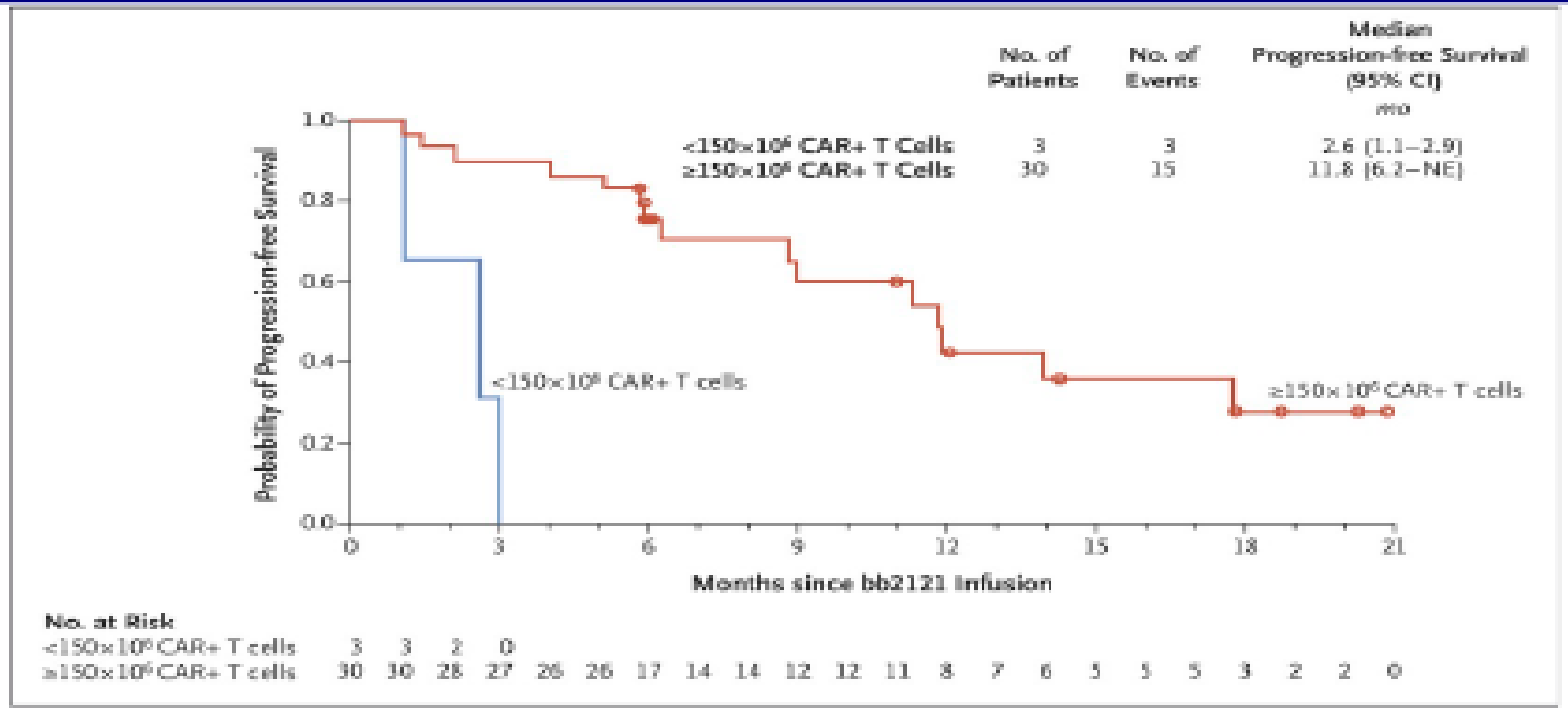
Bb2121 responses



Raje et al. *The New England Journal of Medicine*, 2019

Progression free survival

Bb2121 progression-free survival

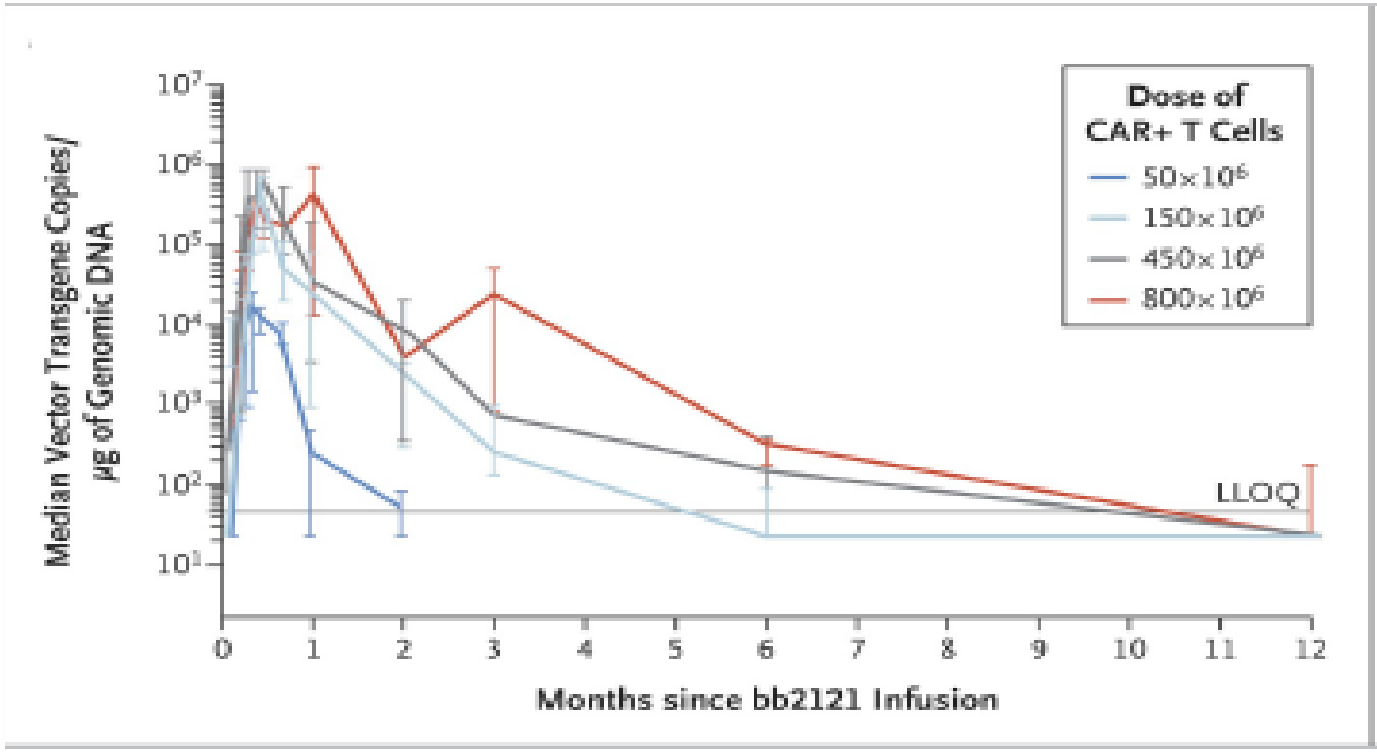


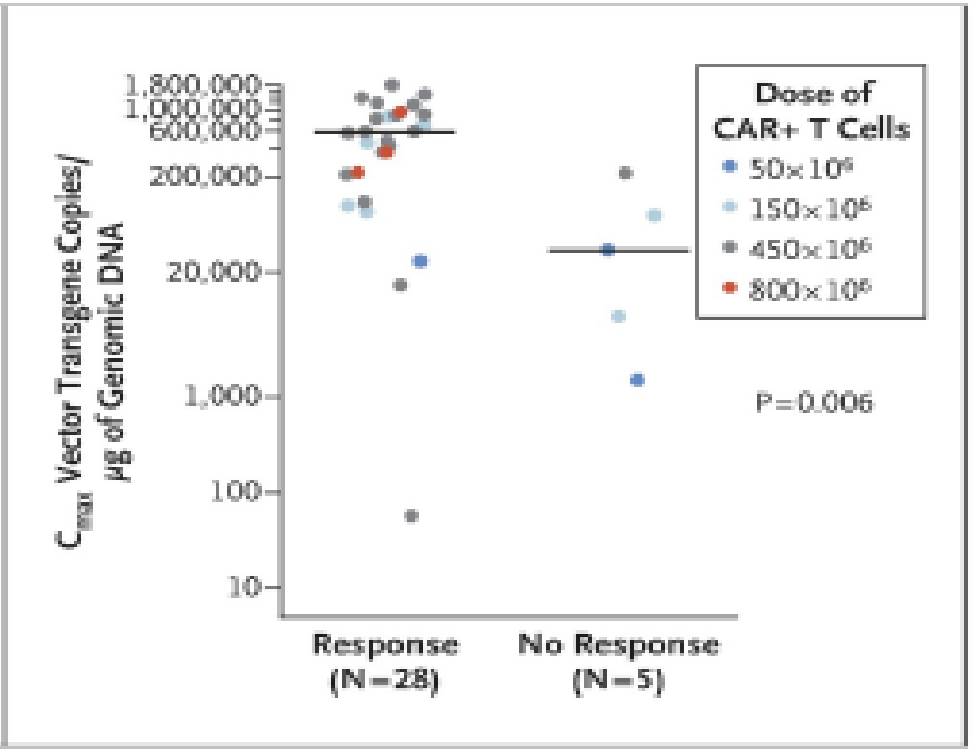
- Cytokine-release syndrome was relatively mild; 2 of 33 patients had Grade 3, and none had Grade 4 CRS
- Only 1 of 33 patients had Grade 3 or 4 neurologic toxicity
- 7 patients received tocilizumab and 4 received corticosteroids

Raje et al. *The New England Journal of Medicine*, 2019

Association with response

Bb2121 CAR T-cell levels were associated with response





Raje et al. *The New England Journal of Medicine*, 2019

Potential advantages

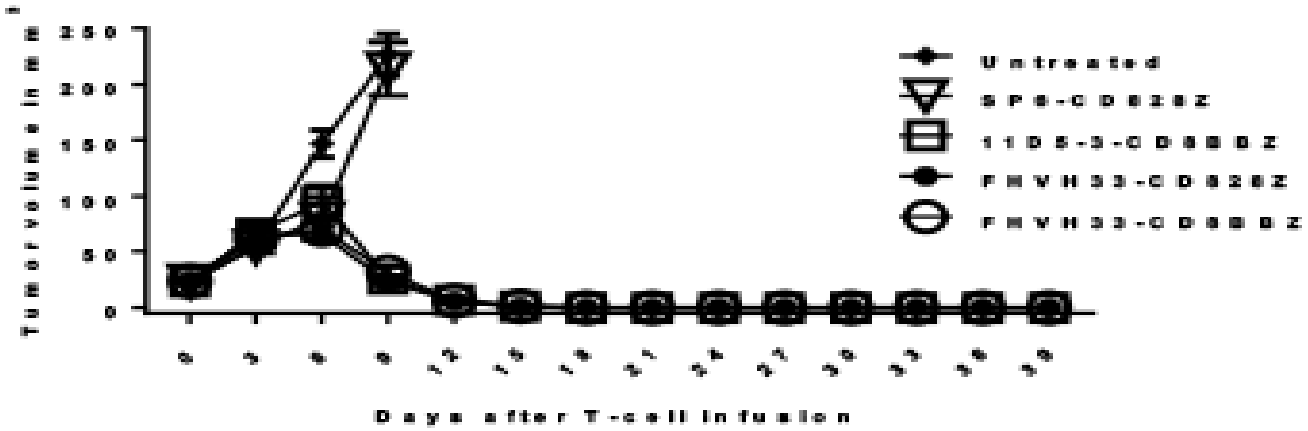
Potential advantages of CARs with heavy-chain-only binding domains led us to develop fully-human heavy-chain-only CARs targeting BCMA

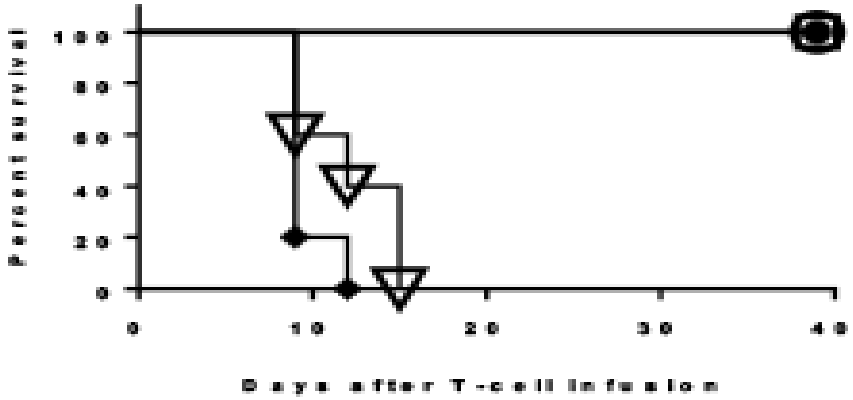
- FHVH: Fully-human heavy chain variable domain generated in a transgenic rat by TeneoBio, Inc.
- Because the heavy-chain-only domains do not have linkers, immune responses directed at linkers and junctions between the linker and variable domains are eliminated.
- Heavy-chain-only binding domains are smaller (good for bispecific CARs).
- In vitro, FHVH33-CD8BBZ function was equivalent to function of a CAR with the 11D5-3 murine scFv used in several clinical trials.

Heavy chain only CARs

Heavy-chain-only CARs eradicated established tumors from mice

- Immunodeficient NSG mice were used
- RPMI8226 tumors were established
- 10 days after tumor injection, mice were injected intravenously with $2x10^6$ CAR^+ T cells

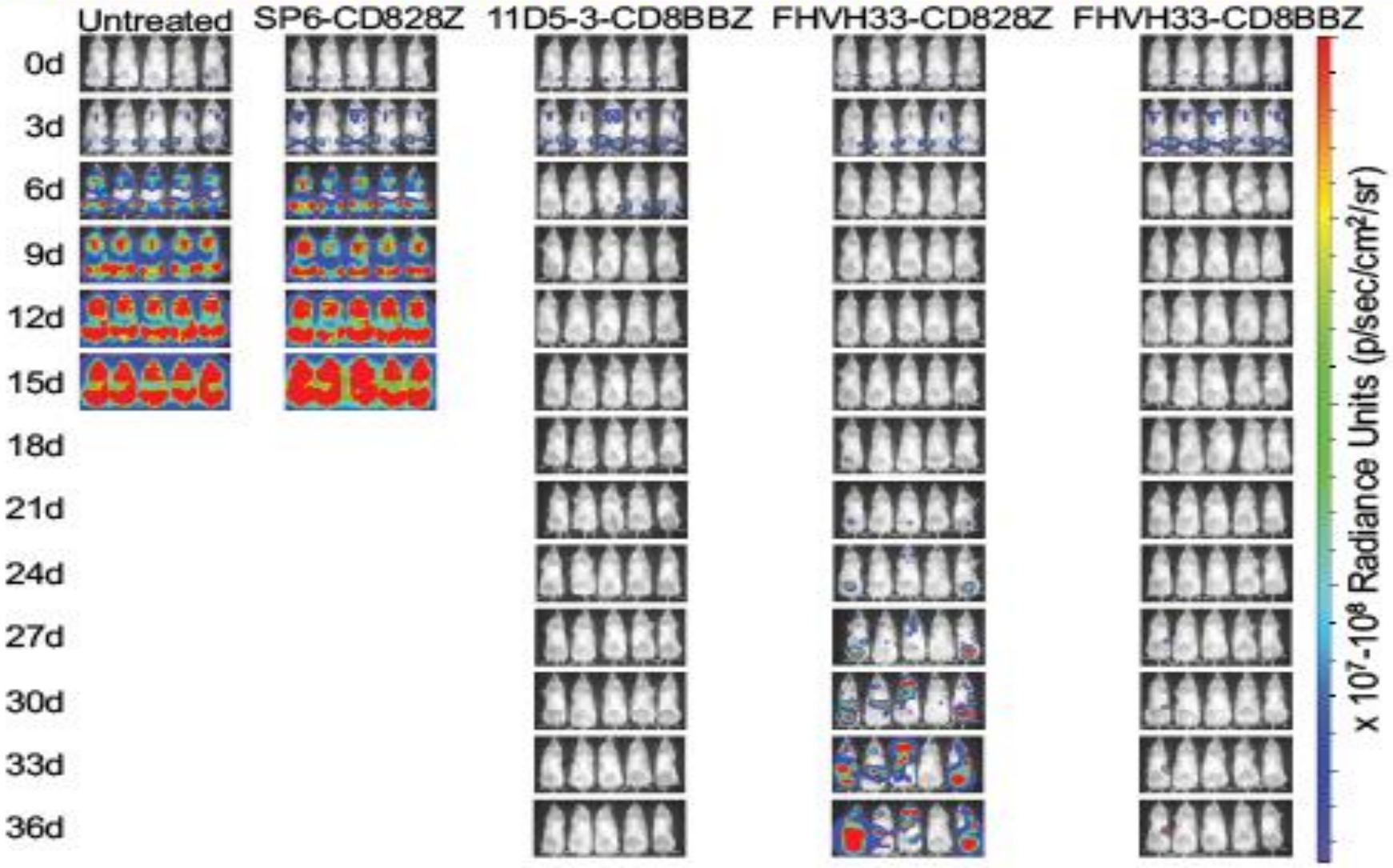




FHVH33-CD828Z CAR T cells

FHVH33-CD8BBZ CAR T cells can eliminate disseminated malignancy

- NSG mice were injected IV with MM.1S cells.
- 10 days later, they were treated with $1x10^6$ CAR T cells
- Malignancy was eradicated by T cells expressing each of 3 anti-BCMA CARs:
  - 11D5-3-CD8BBZ
  - FHVH33-CD828Z
  - FHVH33-CD8BBZ
- Remissions were short in mice getting FHVH33-CD828Z T cells



Clinical trial

Clinical trial of FHVH33-CD8BBZ T cells

Eligibility

- Enrolling relapsed multiple myeloma
- Patients need normal cardiac ejection fraction, no history of cardiac problems
- Creatinine maximum 1.5 mg/dL
- Platelets minimum 55/μL
- Must have measurable multiple myeloma

Trial design

- Dose escalation
- Conditioning regimen of 300 mg/m^2 cyclophosphamide and 30 mg/m^2 fludarabine daily for 3 days
- One infusion of anti-BCMA CAR T cells 3 days after the chemotherapy ends

Summary of responses

Summary of responses of anti-BCMA CAR T at all dose levels

CAR T-cell dose/kg	Response (duration in weeks, + means ongoing)
$0.75x10^6$	sCR (24), PR (25+), PR (48+), sCR (42+), VGPR (31), PR (2, died of influenza)
$1.5x10^6$	PR (8), SD (4), PR (2)
$3x10^6$	VGPR (24+), VGPR (11), PR (9+)
$6x10^6$	PD (due to high dose steroids), PR (8+), PR (4+)

Patients received no anti-myeloma therapy after infusion of CAR T cells until progression occurred

- Median age 63 and a median of 6 prior lines of therapy in patients treated so far.

Signaling lymphocyte activation molecule 7

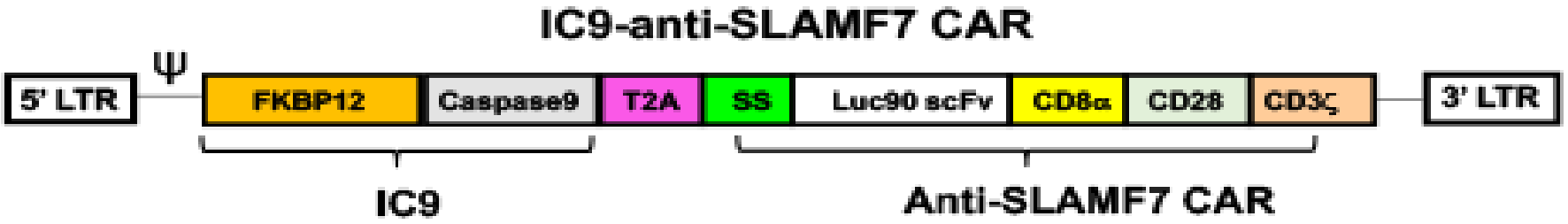
Hypothesis: Signaling lymphocytic activation molecule 7 (SLAMF7) is an appropriate target for CAR T cells

- Rationale: BCMA loss has been documented in clinical trials of anti-BCMA CARs, so new target antigens are needed.
- SLAMF7 is highly expressed on multiple myeloma cells.
- SLAMF7 is expressed on most NK cells and some $CD8^+$ T cells along with small fractions of monocytes and $CD4^+$ T cells.
- Because of expression on many types of leukocytes, a suicide gene is needed in case of chronic cytopenias.
- The most clinically-tested suicide gene is inducible caspase 9 (IC9) that is activated with Rimiducid (Di Stasi et al. *New England Journal of Medicine* 2011).

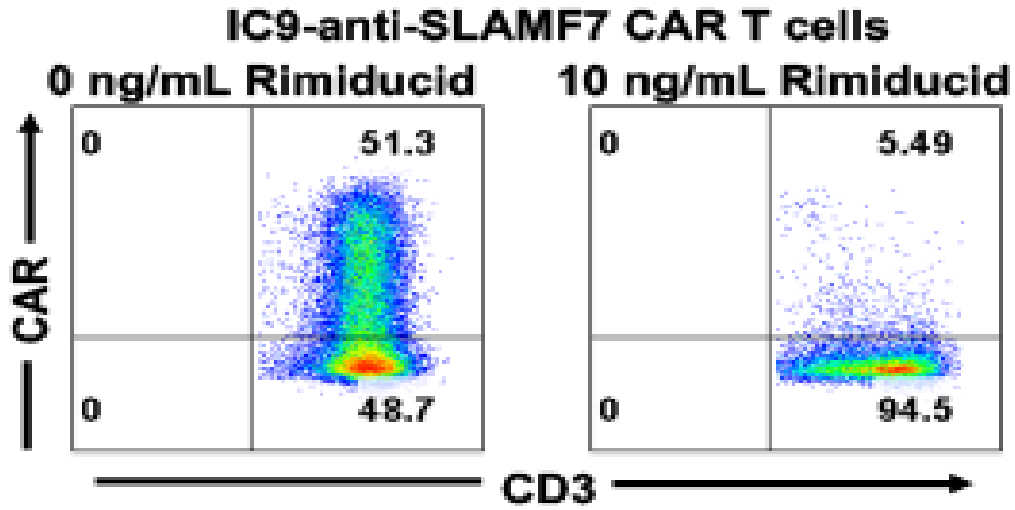
Suicide gene

Construct encoding both a suicide gene and an anti-SLAMF7 CAR

- We designed a CAR construct composed of IC9 and an anti-SLAMF7 CAR connected by a T2A ribosomal skip sequence.
- The construct causes expression of both proteins in the same cell.



Rimiducid exposure for 6 hours in vitro activates suicide gene



Summary

Summary and future plans for CAR T-cell therapies of multiple myeloma

- We demonstrated for the first time that anti-BCMA CAR T cells have powerful activity against multiple myeloma.
- Anti-BCMA CAR T cells are in international phase II clinical trials, but multiple myeloma is phenotypically heterogeneous, so targeting more than 1 antigen is very important.
- Heavy-chain binding domains offer potential advantages of reduced immunogenicity and reduced size compared with traditional scFvs.
- SLAMF7 is a promising but untested target for CAR T cells.
- More multiple myeloma-associated antigens are needed.

Future plans

Summary and future plans for CAR T-cell therapies of multiple myeloma

- Anti-BCMA CAR T cells have powerful activity against multiple myeloma.
- Anti-BCMA CAR T cells are in international phase II clinical trials, but multiple myeloma is phenotypically heterogeneous, so targeting more than 1 antigen is important.
- More multiple myeloma antigens are needed in addition to BCMA
- Currently at the NCI, we have an actively-recruiting trial of an anti-BCMA CAR with a heavy-chain-only antigen recognition domain.
- We have treated the first 2 patients on the SLAMF7 trial, and we are searching for more patients.

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bb2121
Celgene collaborators
CRB401 investigators